Concentration Calculation

Lyophilized peptides may contain anywhere from 10% to as much as 70% bound water and salts by weight. More hydrophilic peptides generally contain more bound water and salts compared to hydrophobic peptides. Therefore, it is difficult to ascertain the actual peptide concentration based on the weight of the lyophilized peptide. If the peptide has a chromophore in the sequence (W or Y residues), peptide concentration can be conveniently determined based on the extinction coefficient of these residues. The following steps can be used for the calculations:

1. Molar extinction coefficients of chromophoric residues at 280 nm at neutral pH using a 1-cm cell:
Tryptophan 5560 AU/mmole/ml
Tyrosine 1200 AU/mmole/ml

2. The extinction coefficient of each chromophore in the peptide sequence is generally considered to be additive, that is, the overall molar extinction coefficient of the peptide depends on the types and number of these choromophoric residues in the sequence.

3. Calculations: mg peptide per ml = (A280 x DF x MW) / e
where A280 is the actual absorbance of the solution at 280 nm in a 1-cm cell, DF is the dilution factor, MW is the molecular weight of the peptide and e is the molar extinction coefficient of each chromophore at 280 nm

4. **Hypothetical example:** A 50X diluted solution of a peptide with the sequence RRNWNQYKIQFGYSFSNSE (MW = 2414) reads 0.5 AU at 280 nm in a 1-cm cell. To calculate the original peptide concentration in the stock peptide solution:
Mg peptide/ml = (0.5AU x 50 x 2414 mg/mmole) [(1 x 5560) + (2 x 1200)] AU/mmole/ml = 7.58

5. Cautions:

- Any absorbance calculation assumes that the peptide is unfolded and the chromophores are exposed, which is usually the case in short, soluble peptides. If there are doubts about the solubility or the folding of the peptide, it is advisable to make the measurement under denaturing conditions (e.g., 6M GdnHCl or 8M urea). Obviously, these peptide solutions will be rendered useless, unless the denaturants are removed.
- If the sequence does not have Trp or Tyr, the only practical option is to do amino acid analysis.